Counting - Luna

1. Take a look at the cells and see that they are alive and well.
2. Heat ~3 ml trypsin (15ml falcon in the freezer) in a 37^0c bath for ~10 minutes. Write the date on the falcon.
3. Heat 10 ml medium (6 for inactivation of trypsin, 2 four counting, 2 extra) in a 37^0c bath for ~10 minutes.
4. Aspirate old medium and immediately add ~3 ml trypsin and incubate for ~3 minutes in 37^0c.
5. After ~3 minutes, take a look at the cells and check if they detached. If not, continue incubation.
6. Add 6 ml medium to the old flask with the trypsin, mix and move entire volume to an empty falcon.
7. Centrifuge @ room temperature for 5 minutes.
8. In the meantime, prepare 2 eppendorfs. Add 90 µl medium to one and leave the other empty.
9. Carefully Remove falcon from centrifuge, aspirate medium and resuspend well in 1 ml medium.
10. Immediately after resuspending, move 10 µl to the 90 µl eppendorf and mix well.
11. Take 10 µl from the 90 µl eppendorf and move them to the empty eppendorf.
12. Add 10 µl trypan blue. **Trypan blue is very toxic**! Mix well, take 10 µl and apply it to the glass.
13. Insert glass to the machine.
14. Adjust focus and press count.

Counting - Traditional

1. Take a look at the cells and see that they are alive and well.
2. Heat ~3 ml trypsin (15ml falcon in the freezer) in a 37^0c bath for ~10 minutes. Write the date on the falcon.
3. Heat 10 ml medium (6 for inactivation of trypsin, 2 four counting, 2 extra) in a 37^0c bath for ~10 minutes.
4. Aspirate old medium and immediately add ~3 ml trypsin and incubate for ~3 minutes in 37^0c.
5. After ~3 minutes, take a look at the cells and check if they detached. If not, continue incubation.
6. Add 6 ml medium to the old flask with the trypsin, mix and move entire volume to an empty falcon.
7. Centrifuge @ room temperature for 5 minutes.
8. In the meantime, prepare 2 eppendorfs. Add 990 µl medium to one and leave the other empty.
9. Carefully Remove falcon from centrifuge, aspirate medium and resuspend well in 1 ml medium.
10. Immediately after resuspending, move 10 µl to the 990 µl eppendorf and mix well.
11. Take 20 µl from the 990 µl eppendorf and move them to the empty eppendorf.
12. Add 20 µl trypan blue. Trypan blue is very toxic! Mix well, take 20 µl and apply it to the glass.
13. Count at least 5 squares. Average and multiply by million.